are millimolar divalent cations present in the extracellular milieu, it is likely that most extracellular ATP released from synaptic vesicles is chelated by divalent. We found that some subtypes of P2X receptors can be activated by both free and divalent-bound ATP, while others can only be efficiently activated by free ATP. This subtype specific activation by different forms of ATP parallels the pharmacological sensitivity to other agonists and antagonists, pointing to the existence of two distinct classes of ligand binding pockets. We are currently examining which forms of ATP activate heteromeric P2X receptor channels formed by subunits with different sensitivity to divalent-bound ATP.

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Functional P2X7 Receptor Expression in the Magnocellular Neurons of the Hypothalamic Neurohypophysial System (HNS)

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Our laboratory has presented evidence for P2x7 receptors (P2x7R) in isolated terminals of the neurohypophysis (NH) (Cuadra et al., 2010, *Biophys. J.***97***:* 1474). In this study we further examined P2x7R immunoreactivity (IR) and function. A stereo-specific ecto-P2x7R antibody was used for IR studies (Kim el al., 2001, JBC, **276**:23262) in the rat HNS: supraoptic nucleus (SON), paraventricular nucleus (PVN) and NH. We found limited P2x7R IR in the SON and the PVN, which was absent from the somata of OT- and AVP-neurons. P2x7R IR was co-localized in some cell bodies containing the glial specific marker, GFAP. In contrast, P2x7R IR was abundantly seen on the membrane of NH terminals (NHT), with dense IR in terminals lining the capillary borders. This was confirmed in isolated NHT that showed IR puncta on the membranes of both AVP- and OT-containing terminals. Initial results using ratiometric calcium imaging with Fura-2 in isolated NHT showed differential $[Ca^{2+}]_i$responses to 100 μM *vs*. 1 mM ATP; some NHT responded to both doses while others responded only to 1 mM ATP. A similar distribution in response was observed with ATP generated ion currents during patch clamp recordings. NHT responded with either high sensitivity (HS) or low sensitivity (LS) to ATP. The HS group corresponded to AVP-NHT, which is reported to express P2x2R, P2x3R, P2x4R and P2x7R (Knott et al., 2005, Pfluger Arch, **405**:381). In contrast, the LS group corresponded to OT-NHT, which is consistent with a P2x7R response. Together these data suggest that in the HNS P2x7R is expressed chiefly in the AVP- and OT-secreting terminals of magnocellular neurons. (Supported by NIH grant NS29470 to JRL)

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Understanding the Kinetics of ATP-Activated P2X2A and P2X2B Receptor Channels using a Markov State Model

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ATP-gated P2X2 receptors exhibit two opposite activation-dependent changes during sustained agonist application, pore dilation and pore closing (desensitization), through a process that is incompletely understood. To address this issue and to clarify the roles of Ca^{2+} and the C-terminal domain in gating, we combined biophysical and mathematical approaches using the full size receptor (labeled P2X2aR) and the splice form missing 69 residues in the C-terminal domain (labeled the P2X2bR). Both forms of the receptor developed conductivity for large organic cations within 2-6 s of ATP application and desensitized in a Ca^{2+} influx-dependent manner, whereas P2X2bR also desensitized in a Ca^{2+} influx-independent manner. In whole-cell recording with broken membranes, we also observed use-dependent facilitation of desensitization, reflecting the altered Ca^{2+} handling by cells. Such behavior was accounted for by a Markov state kinetic model with 12 states describing the ATP binding/unbinding and activation/desensitization. The model assumes that naïve receptors open when two ATP molecules bind and slowly dilates to a higher conductance state when a third ATP binds, generating a shift to less negative reversal potential observed experimentally in organic cation-containing medium. The use-dependent desensitization is modeled by a Ca^{2+}-dependent toggle switch, whereas the P2X2bR model also exhibits fast Ca^{2+}-independent desensitization. The model is extended to include memory to previous stimulations that not only explained the decrease in the slope of the IV-curves during −80 to +80 voltage ramps delivered twice per second, but also captured the effect of ATP stimulation when cells were held at positive holding potential.

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The Effect of Anions on the Human P2X7 Receptor

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P2X7 receptors (P2X7Rs) are nonselective cation channels that are opened by the binding of extracellular ATP and are involved in the modulation of epithelial secretion, inflammation and nociception. Here, we investigated the effect of extracellular anions on channel gating and permeation of human P2X7Rs (hP2X7Rs) expressed in *Xenopus laevis* oocytes. Two-microelectrode voltage-clamp recordings showed that ATP-induced hP2X7R-mediated currents increased when extracellular chloride was substituted by the organic anions glutamate or aspartate and decreased when chloride was replaced by the inorganic anions nitrate, sulfate or iodide. ATP concentration-response comparisons revealed that substitution of chloride by glutamate decreased agonist efficacy, while substitution by iodide increased agonist efficacy at high ATP concentrations. Meanwhile, the ATP potency remained unchanged. Activation of the hP2X7R at low ATP concentrations via the high-affinity ATP effector site was not affected by the replacement of chloride by glutamate or iodide. To analyze the anion effect on the hP2X7R at the single-molecule level, we performed single-channel current measurements using the patch-clamp technique in the outside-out configuration. Chloride substitution did not affect the single-channel conductance, but the probability that the P2X7R channel was open increased when chloride was replaced by glutamate and decreased when chloride was replaced by iodide. This effect was due to an influence of the anions on the mean closed times of the hP2X7R channel. We conclude that hP2X7R channels are not anion-permeable in physiological Na^+-based media and that external anions allosterically affect ion channel opening in the fully ATP^{4-}-liganded P2X7R through an extracellular anion binding site.

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Direct Permeation of the P2X7 Receptor Pore by Nanometer Molecules

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The selectivity of ion channels is generally considered to be fixed. The ATP-gated P2X7 receptor is unusual in that its activation leads to a progressive increase in the permeation of large cations during several seconds (*e.g.* YO-PRO-1). However, controversy remains whether these large molecules directly permeate the P2X7 receptor pore or use some coupled transport mechanism. In HEK293 cells expressing the rP2X7 receptor, simultaneous measurement of ionic current and YO-PRO-1 influx showed they both increased concomitantly with the application of ATP. At positive potentials YO-PRO-1 influx was much reduced, implying that movement of this large cation (1.8 x 0.8 x 0.7 nm) is influenced by the membrane electric field in a manner similar to smaller cations. Increasing the positivity in the pore of the P2X7 receptor significantly enhanced chloride permeability (D352N and T348K; P_{Cl}/P_{Na} increased 10-fold compared to WT). The selective permeability of large molecules was then measured using two ions of similar size and structure, bearing either a positive or a negative net charge: both the cation (ethidium: 1.2 x 1.0 x 0.5 nm) and the anion (FITC: 1.3 x 1.1 x 0.7 nm) were simultaneously detected by intracellular fluorescence. Introduction of a positive charge in the pore (T348K) or removal of a negative charge (D352N) increased the influx of the anion FITC, and decreased the influx of the cation ethidium. These effects parallel the findings of chloride permeability, demonstrating that by increasing the positivity in the pore of the P2X7 receptor the permeation pathway is more energetically favourable for anions, whether they are small or large. This implies that large molecules directly permeate the P2X7 receptor pore and any model of channel opening should accommodate a pore diameter >1 nm.

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The Gating Mechanism of a P2X4 Receptor: Normal Mode Analysis and Molecular Dynamics Simulations

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P2X4 receptors are trimeric ATP-gated non-selective cation channels which play crucial roles in various physiological processes. It remains unclear how ATP binding triggers channel opening. Here, we propose a gating mechanism